invitrogen™

pYES2.1-E and pYC2-E Echo™-Adapted Expression Vectors

For cloning of the gene of interest using the Echo™ Cloning System and expression in *Saccharomyces cerevisiae*

Catalog nos. ET200-XX, ET210-XX

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User Manual

Table of Contents

Kit Contents and Storage iv
Accessory Products vii

Introduction 1
Overview 1

Methods 5
Recombining Your Gene into pYES2.1-E or pYC2-E 5
Transforming the Recombination Reaction 6
Yeast Transformation 10
Expression of Your Recombinant Protein 12

Appendix 16
Small-Scale Yeast Transformation 16
Recipes 17
Maps of pYES2.1-E and pYC2-E 21
Features of pYES2.1-E and pYC2-E 23
Map of pYES2.1-E/Uni-*lacZ* 24
Map of pYC2-E/Uni-*lacZ* 25
Technical Service 26
Purchaser Notification 27
Product Qualification 28
References 29

Kit Contents and Storage

Shipping and Storage

The pYES2.1-E or pYC2-E Echo™-Adapted Expression Vectors are shipped on dry ice. The INVSc1 stab is shipped at room temperature. Upon receipt, store the reagents as follows:

- pYES2.1-E or pYC2-E reagents at -20°C
- One Shot® Chemically Competent *E. coli* at -80°C
- INVSc1 stab at +4°C

Types of Kits

Several pYES2.1-E and pYC2-E Echo™ Cloning System Kits are available. The table below lists the kits that include the pYES2.1-E or pYC2-E Echo™-Adapted Expression Vectors.

Kit	Reagents Supplied	Catalog nos.
pYES2.1-E Echo™-Adapted Expression Vector Kit	pYES2.1-E vector Expression Control vector Cre Recombinase 10X Recombinase buffer T7 Forward Sequencing Primer INVSc1	ET200-01
pYC2-E Echo™-Adapted Expression Vector Kit	pYC2-E vector Expression Control vector Cre Recombinase 10X Recombinase buffer T7 Forward Sequencing Primer INVSc1	ET210-01
pYES2.1-E Echo™-Adapted Expression Vector Kit with a choice of Donor Vector Kit and One Shot® TOP10 Chemically Competent *E. coli* (see page viii for more information on donor vectors)	pUni/V5-His TOPO TA Cloning® Kit	ET200-10C
	pUniBlunt/V5-His TOPO® Cloning Kit	ET200-20C
	pUni/V5-His A, B, and C	ET200-30C
	pUniD/V5-His TOPO® Cloning Kit	ET200-40C
pYC2-E Echo™-Adapted Expression Vector Kit with a choice of Donor Vector Kit and One Shot® TOP10 Chemically Competent *E. coli* (see page viii for more information on donor vectors)	pUni/V5-His TOPO TA Cloning® Kit	ET210-10C
	pUniBlunt/V5-His TOPO® Cloning Kit	ET210-20C
	pUni/V5-His A, B, and C	ET210-30C
	pUniD/V5-His TOPO® Cloning Kit	ET210-40C

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Kit Contents and Storage, continued

pYES2.1-E and pYC2-E Reagents

pYES2.1-E and pYC2-E reagents are listed below. Store at -20°C.

Item	Concentration	Amount
pYES2.1-E or pYC2-E	Supercoiled, lyophilized in TE, pH 8	20 µg
Cre recombinase	Please check the label on the tube for exact concentration of the enzyme Enzyme supplied in: 50 mM Tris-HCl, pH 8.0 5 mM EDTA 1 mM EGTA 10 mM β-mercaptoethanol 20% Glycerol	15 µl
10X Recombinase Buffer	500 mM Tris-HCl, pH 7.5 100 mM $MgCl_2$ 300 mM NaCl 1.0 mg/ml BSA	25 µl
T7 Forward Sequencing Primer (20 mer)	Lyophilized in TE Buffer, pH 8 (5´-TAATACGACTCACTATAGGG-3´)	2 µg (327 pmoles)
Expression control (pYES2.1-E/Uni-*lacZ* or pYC2-E/Uni-*lacZ*)	Supercoiled, lyophilized in TE, pH 8	20 µg
INVSc1	Yeast strain	1 stab

INVSc1 *S. cerevisiae* Strain

INVSc1 is a fast-growing, easily transformed diploid strain. Store at +4°C.

Genotype:

MATα his3Δ1 leu2 trp1-289 ura3-52
MATa his3Δ1 leu2 trp1-289 ura3-52

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Kit Contents and Storage, continued

One Shot® TOP10 *E. coli*

The table below describes the items included in the One Shot® TOP10 Chemically Competent *E. coli* kit. **Store at -80°C**.

Item	Composition	Amount
SOC Medium (may be stored at room temperature or +4°C)	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 ml
TOP10 *E. coli*	--	11 x 50 µl
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

Genotype of TOP10

Use this strain for transformation of the fusion vector. **Note:** This strain cannot be used for transformation and growth of donor vectors.

F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

Accessory Products

Products Available Separately

Many of the reagents in the pYES2.1-E and pYC2-E Echo™-Adapted Expression Vector Kits, and additional reagents that may be used with the Echo™ Cloning System are available separately. In addition, reagents for expression, detection, and purification of your protein of interest from your pYES2.1-E or pYC2-E fusion vector are available from Invitrogen. Ordering information is provided below.

Transformation Kit

The *S.c.* EasyComp™ Transformation Kit (Catalog no. K5050-01) is designed for rapid preparation of transformation-competent *Saccharomyces cerevisiae* cells.

Echo™ Cloning Products

Many of the reagents supplied in the pYES2.1-E or pYC2-E Echo™-Adapted Expression Vectors, as well as additional reagents that may be used for Echo™ Cloning, are available separately from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog No.
T7 Forward Primer	2 µg	N560-02
One Shot® TOP10 Chemically Competent *E. coli*	21 x 50 µl	C4040-03
One Shot® PIR1 Chemically Competent *E. coli*	11 x 50 µl	C1010-10
One Shot® PIR2 Chemically Competent *E. coli*	11 x 50 µl	C1111-10
Cre Recombinase	10 reactions	R100-10

Continued on next page

Accessory Products, continued

Donor Vectors

The table below lists a variety of donor vectors currently available from Invitrogen to facilitate cloning of your gene of interest for use with Echo™ Cloning System.

Product	Application	Quantity	Catalog no.
pUniD/V5-His-TOPO® Cloning Kit	Directional cloning of blunt PCR products	10 reactions	ET004-10
pUni/V5-His-TOPO TA Cloning® Kit	Cloning A-tailed PCR products	10 reactions	ET001-10
pUniBlunt/V5-His-TOPO® Cloning Kit	Cloning blunt PCR products	10 reactions	ET002-10
pUni/V5-His A, B, and C	Cloning DNA fragments using restriction enzymes	10 reactions	ET003-10

Detection and Purification

After expressing your protein of interest from your pYES2.1-E or pYC2-E fusion vector, use the reagents below to detect and purify your protein of interest.

Product	Amount	Catalog No.
Anti-His(C-term) Antibody	50 µl*	R930-25
Anti-His(C-term)-HRP Antibody	50 µl*	R931-25
Anti-V5 Antibody	50 µl*	R960-25
Anti-V5-HRP Antibody	50 µl*	R961-25
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Metal-Binding Resin (precharged resin provided as a 50% slurry in 20% ethanol)	50 ml	R801-01
	150 ml	R801-15
Purification Columns (10 ml polypropylene columns)	50	R640-50

*Enough for 25 Westerns.

Introduction

Overview

Introduction

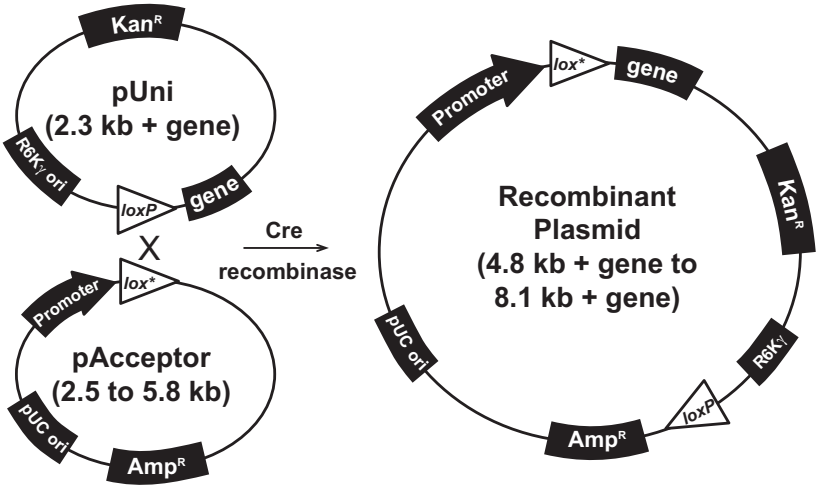
The Echo™ Cloning System allows direct recombination of your gene of interest downstream of an appropriate promoter for expression in the host system of choice. The vectors pYES2.1-E and pYC2-E are specifically designed for regulated expression of recombinant proteins in *S. cerevisiae*. The *GAL1* promoter regulates expression in yeast.

Echo™ Cloning System

The Echo™ Cloning System is based on the univector plasmid-fusion system (UPS) described by Elledge and coworkers to quickly and easily recombine a gene of interest into a series of recipient (acceptor) vectors (Liu *et al.*, 1998; Liu *et al.*, 1999). The system consists of the univector (donor vector) containing the gene of interest and recipient (acceptor) vectors containing various regulatory sequences for expression in the host of choice. The system utilizes the *cre-lox* site-specific recombination system of bacteriophage P1 to recombine the gene of interest into the acceptor vector of choice (Abremski *et al.*, 1983; Sternberg *et al.*, 1981). The product of the *cre* gene is a site-specific recombinase that catalyzes recombination between two 34 bp *loxP* sequences to resolve P1 dimers generated by replication of circular lysogens.

Plasmid Fusion

The donor vector (pUni) and the acceptor vector (i.e. pYES2.1-E or pYC2-E) each contain a single *lox* site. The donor vector contains a *loxP* site while pYES2.1-E and pYC2-E each contain a *loxH* site (for more information on *loxP* and *loxH*, please see the next page). Construct the donor vector containing the gene of interest via the TOPO® Cloning method or traditional restriction enzyme-mediated cloning (see page viii). pYES2.1-E and pYC2-E contain the appropriate transcription regulatory sequences that will control expression of the gene of interest in *Saccharomyces*. A unique *loxH* site is located downstream of these sequences in both vectors. By mixing the donor vector containing the gene of interest with pYES2.1-E or pYC2-E in the presence of Cre recombinase, a plasmid fusion is created that expresses the gene of interest in *Saccharomyces*. A generic diagram is shown below.



*lox** = *loxP* or *loxH* depending on acceptor vector

Continued on next page

Overview, continued

loxP or *loxH* Sites

The sequence of the *loxP* site is shown below. The *loxP* site consists of a 34 bp sequence containing two 13 bp inverted repeats (see underlined bases) separated by an 8 bp spacer (Hoess *et al.*, 1982). The inverted repeats may form a stem and loop structure that may reduce expression of the gene of interest in some cases. A variation of the *loxP* site (*loxH*, see below) was created to eliminate the formation of a stem and loop structure and improve expression. Mutated bases are shown in boldface. Please note that some acceptor vectors including pYES2.1-E and pYC2-E contain a *loxH* site. Cre-mediated recombination can still occur between a *loxP* and a *loxH* site although the efficiency may be slightly reduced.

- *loxP*: ATA ACT TCG TAT AGC ATA CAT TAT ACG AAG TTA T
- *loxH*: AT**T** AC**C** TC**A** TAT AGC ATA CAT TAT ACG AAG TTA T

Cre Recombinase

Cre recombinase (MW = 35 kDa) is a site-specific recombinase that binds to specific sequences (*loxP* sites), brings together the target sites, cleaves, and covalently attaches to the DNA. Recombination occurs following two pairs of strand exchanges and ligation of the DNAs in a novel (recombinant) form. A nucleophilic hydroxylated tyrosine initiates the DNA cleavage event by attack on a specific phosphodiester bond followed by the covalent attachment of the recombinase to the target sequence through a phosphoamino acid bond (Abremski and Hoess, 1992; Argos *et al.*, 1986). The reaction does not require any host factors or ATP, but does require Mg^{2+} or spermidine for activity (Abremski *et al.*, 1983). Recombination between two supercoiled substrates, each containing a *loxP* site, results in a supercoiled dimer. The extent of the reaction is 10-20% under optimal conditions (Abremski and Hoess, 1984; Abremski *et al.*, 1983).

Selection of Recombinants

By fusing the two plasmids, kanamycin resistance from the donor vector is now linked to the pUC origin of replication. The recombination reaction is transformed into TOP10 *E. coli* and recombinants selected by plating the transformation reaction onto plates containing kanamycin. Because the donor plasmid carries the R6Kγ origin of replication, it will not propagate in TOP10. In addition, the acceptor vector, which carries the ampicillin resistance gene, will not be selected. Therefore every colony that is selected on kanamycin will represent a recombined fusion plasmid.

Continued on next page

Overview, continued

pYES2.1-E and pYC2-E

pYES2.1-E or pYC2-E allow you to induce expression of your protein of interest in *S. cerevisiae*. Both pYES2.1-E and pYC2-E contain the following features:

- Yeast *GAL1* promoter for high-level inducible protein expression in yeast by galactose and repression by glucose (Giniger *et al.*, 1985; West *et al.*, 1984) (see page 12 for more information)
- A *loxH* site for plasmid fusion
- URA3 auxotrophic marker for selection of yeast transformants
- Ampicillin resistance for selection in *E. coli*

The vectors differ in their mechanism of replication.

- pYES2.1-E contains the 2µ origin for episomal maintenance and high copy replication.
- pYC2-E contains the *CEN6*/*ARSH4* sequence for non-integrative centromeric maintenance and low copy replication.

For more information and a map of each vector, please see pages 21-23.

Other acceptor vectors are available separately and are provided with their own manuals. For more information on other available acceptor vectors, please visit our Web site (www.invitrogen.com) or call Technical Service (see page 26).

2µ Origin

The pYES2.1-E vector contains the 2µ origin for maintenance and replication in yeast. The sequence containing the 2µ origin was originally isolated from the naturally-occurring yeast 2µ plasmid (Hartley and Donelson, 1980). When placed in a heterologous expression plasmid (i.e. pYES2.1-E), the presence of the 2µ origin allows the plasmid to be episomally maintained and replicated at high copy number (generally 10-40 copies per cell).

CEN6/*ARSH4* Sequence

The pYC2-E vector contains the *CEN6*/*ARSH4* sequence for maintenance and replication in yeast (Sikorski and Hieter, 1989). The *CEN6*/*ARSH4* sequence is a 518 bp hybrid DNA fragment that contains a yeast centromere sequence (*CEN*) and an autonomously replicating sequence (*ARS*) (Sikorski and Hieter, 1989). The *CEN6* sequence is derived from the *CEN6* locus of yeast chromosome 6 (Panzeri and Philippsen, 1982) while the *ARSH4* sequence is derived from the yeast histone H4-associated *ARS* (Bouton and Smith, 1986). When placed in a heterologous expression plasmid (i.e. pYC2-E), the presence of the *CEN6*/*ARSH4* sequence allows non-integrative centromeric maintenance and low copy number replication of the plasmid (generally 1-2 copies per cell).

Continued on next page

Overview, continued

Experimental Outline

The table below describes the general steps needed to recombine, transform, and express your protein of interest.

Step	Action	Page
1	Perform the recombination reaction using your donor vector and pYES2.1-E or pYC2-E.	5
2	Transform the recombination reaction into competent TOP10 *E. coli* and select recombinants on LB plates containing 50 μg/ml kanamycin.	6
3	Pick transformants and analyze by restriction digestion.	7
4	Isolate plasmid DNA, transform into INVSc1, and select for uracil prototrophy.	10-11
5	Induce with galactose to express your gene of interest.	12-13
6	Assay for expression of your protein.	14
7	Purify your protein, if desired.	15

Methods

Recombining Your Gene into pYES2.1-E or pYC2-E

Introduction

At this point you should have a plasmid preparation of your donor vector in addition to pYES2.1-E or pYC2-E. Please review the information below and on the next page before performing the recombination reaction.

Preparing pYES2.1-E or pYC2-E

To prepare pYES2.1-E or pYC2-E for use, add 20 µl of sterile, deionized water to the lyophilized plasmid. This will yield a 1 µg/µl stock solution. You can further dilute a small aliquot or use as is. Store at -20°C when you are finished.

If you wish to propagate this plasmid or prepare more plasmid DNA, you may transform this plasmid into TOP10 *E. coli* as described on pages 6-7. Use 10-100 ng plasmid for transformation and select on LB plates containing 50-100 µg/ml ampicillin.

Before Starting

You will need the following reagents and equipment.

- 100 ng of your donor vector
- 100 ng of pYES2.1-E or pYC2-E
- Microcentrifuge tubes
- Heat blocks set at 37°C and 65°C
- Ice bucket with ice
- Cre recombinase (included in the kit)
- 10X Recombinase buffer (included in the kit)

Recombination Reaction

1. Set up each 20 µl recombination reaction **on ice** as follows.

Donor vector (100 ng)		x µl
pYES2.1-E or pYC2-E (100 ng)		y µl
10X Recombinase buffer		2 µl
Deionized water	add to a total volume of	19 µl
Cre recombinase		1 µl
Final volume		20 µl

2. Incubate at 37°C for 20 minutes.
3. Incubate at 65°C for 5 minutes to inactivate the recombinase.
4. Place tube on ice and proceed to **Transformation**, next page. If you run out of time, you may store the recombination reaction at +4°C or -20°C overnight. Longer storage times have not been tested.

Transforming the Recombination Reaction

Introduction

Once you have performed the recombination reaction you are ready to transform your *E. coli* host. We recommend TOP10 *E. coli* for transformation but other strains may be used. Strains should be *endA* and *recA* to ensure quality plasmid preparations and reduce the chances of recombination, respectively.

Materials Supplied by the User

In addition to general microbiological supplies (i.e. plates, spreaders), you will need the following reagents and equipment.

- 42°C water bath
- LB plates containing 50 µg/ml kanamycin (see **Important**, below)
- 37°C shaking and non-shaking incubator
- SOC (included in the One Shot® kit)

Important

It is important to select for the fusion plasmid using kanamycin. Remember that the donor vector contains the R6Kγ origin. This origin can only be maintained in *E. coli* strains containing the *pir* gene. By fusing the plasmids, kanamycin is now linked to the pUC origin, allowing the fusion to be maintained in strains that do not contain the *pir* gene (i.e. TOP10). By selecting on kanamycin, you ensure that only colonies that contain the fusion vector are selected.

Preparing for Transformation

The following transformation protocol is for use with the One Shot® TOP10 available with the kit. If you are using other competent cells, please follow the manufacturer's protocol.

For each transformation, you will need one vial of competent cells and two selective plates.

- Equilibrate a water bath to 42°C.
- Thaw the vial of SOC medium from the One Shot® kit and bring to room temperature.
- Warm LB plates containing 50 µg/ml kanamycin at 37°C for 30 minutes.
- Thaw **on ice** 1 vial of One Shot® TOP10 *E. coli* for each transformation.

One Shot® Transformation Reaction

1. Add 5 µl of the recombination reaction to a vial of One Shot® TOP10 *E. coli* and mix gently. **Do not mix by pipetting up and down.**
2. Heat-shock the cells for 30 seconds at 42°C without shaking.
3. Immediately transfer the tubes to ice.
4. Add 500 µl of room temperature SOC medium.
5. Cap the tube tightly and shake the tube horizontally at 37°C for 45 minutes.
6. Spread 50 µl from each transformation on a pre-warmed plate. Pellet the remaining cells and resuspend the cell pellet in 50 µl SOC and plate. Incubate overnight at 37°C.
7. An efficient recombination reaction will produce hundreds of colonies. Pick ~5 colonies for analysis.

Continued on next page

Transforming the Recombination Reaction, continued

Analyzing Positive Clones

1. Culture 5 colonies overnight at 37°C in 2-5 ml LB or SOB medium containing 50 μg/ml kanamycin.
2. Isolate plasmid DNA using your method of choice. If you need ultra-pure plasmid DNA for automated or manual sequencing, we recommend the S.N.A.P.™ MiniPrep Kit (Catalog no. K1900-01) or the S.N.A.P.™ MidiPrep Kit (Catalog no. K1910-01).
3. Analyze the plasmids by restriction analysis. Use an enzyme or enzymes that cut once in the donor vector and once in the acceptor vector to yield two fragments that are distinguishable from one another. Please note that other strategies are possible.
4. (Optional) To sequence the fusion plasmid to confirm the fusion junctions, we recommend using the T7 Forward and Uni1 Forward sequencing primers. Refer to the diagram on the following page for the sequences around the pYES2.1-E or pYC2-E *loxH* site. Refer to the donor vector manual for the sequence around the donor vector *loxP* site.

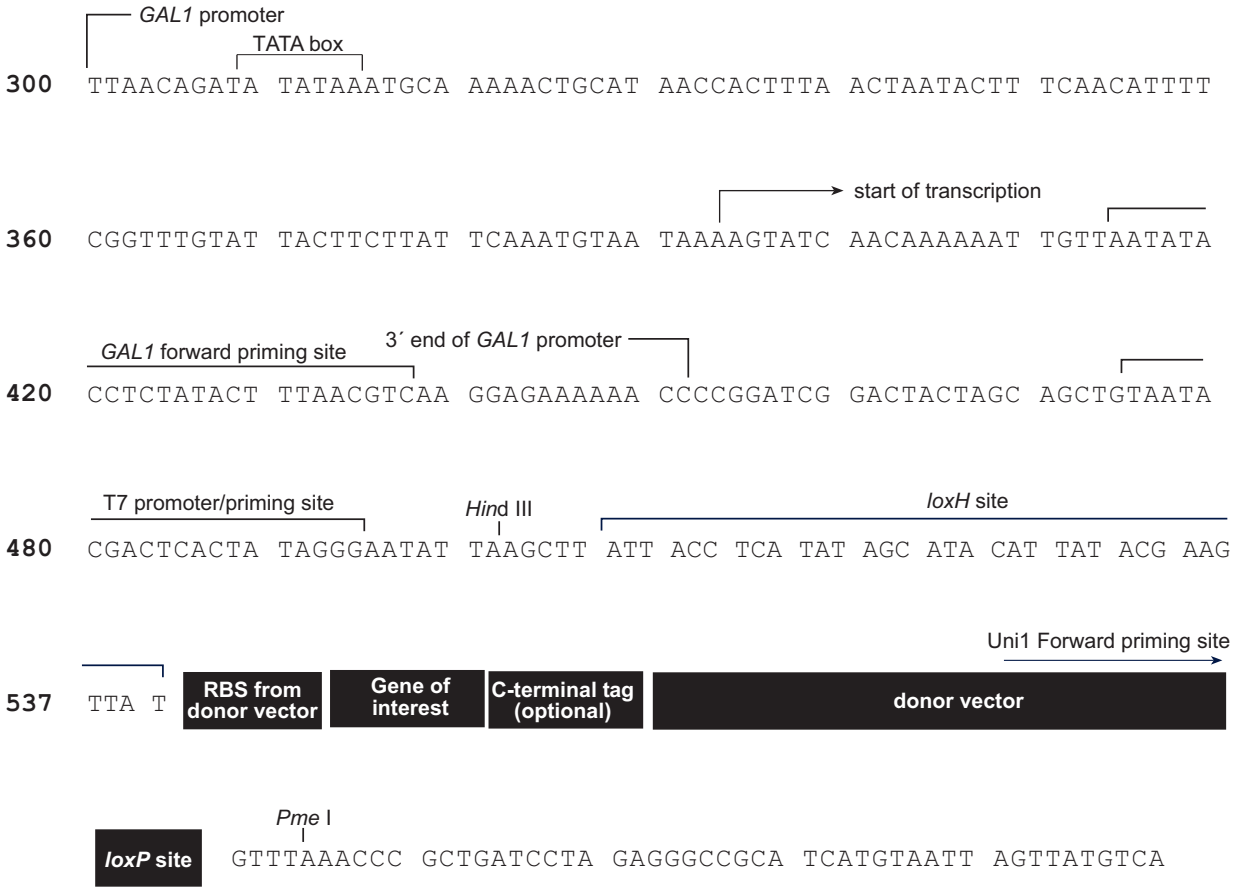
If you need help with setting up restriction enzyme digests or DNA sequencing, refer to general molecular biology texts (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

Continued on next page

Transforming the Recombination Reaction, continued

Sequencing Your Construct in pYES2.1-E or pYC2-E

Aside from the origin of replication, the pYES2.1-E and pYC2-E sequences are identical. Recombination into either vector produces the same sequence upstream of your insert. This sequence is shown below. Unique restriction sites are labeled for your convenience. Note that the complete sequence of pYES2.1-E and pYC2-E can be downloaded from our Web site (www.invitrogen.com) or requested from Technical Service (page 26).


GAL1 promoter
TATA box
300 TTAACAGATA TATAAATGCA AAAACTGCAT AACCACTTTA ACTAATACTT TCAACATTTT
start of transcription
360 CGGTTTGTAT TACTTCTTAT TCAAATGTAA TAAAAGTATC AACAAAAAAT TGTTAATATA
GAL1 forward priming site
3´ end of GAL1 promoter
420 CCTCTATACT TTAACGTCAA GGAGAAAAAA CCCCGGATCG GACTACTAGC AGCTGTAATA
T7 promoter/priming site
HindIII
loxH site
480 CGACTCACTA TAGGGAATAT TAAGCTT ATT ACC TCA TAT AGC ATA CAT TAT ACG AAG
Uni1 Forward priming site
537 TTA T
RBS from donor vector
Gene of interest
C-terminal tag (optional)
donor vector
PmeI
loxP site
GTTTAAACCC GCTGATCCTA GAGGGCCGCA TCATGTAATT AGTTATGTCA

Continued on next page

Transforming the Recombination Reaction, continued

Fusion Vector Analysis

It should be clear from restriction analysis that you have a dimer fusion plasmid consisting of the donor vector and pYES2.1-E or pYC2-E. Occasionally, trimers will result. Trimers usually consist of two donor vector molecules and one acceptor molecule. Please note that trimers usually express as well as the dimer product.

In theory, trimers may result from two sequential fusion events or a single fusion event between a pre-existing monomeric substrate and a dimeric substrate. The production of trimers can be eliminated if gel-purified monomeric supercoiled DNA is used in the recombination reaction.

Preparing Glycerol Stock for Long-Term Storage

Once you have identified the correct clone, prepare a glycerol stock for long-term storage. In addition, store a stock of plasmid DNA at -20°C.

1. Streak out the original colony on LB plates containing 50 µg/ml kanamycin.
2. Select a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml kanamycin.
3. Grow overnight until culture is saturated.
4. Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
5. Store at -80°C.

Yeast Transformation

Introduction

In this section, you will use a small-scale yeast transformation protocol to transform your pYES2.1-E or pYC2-E construct into the INVSc1 yeast host strain included with each vector. After transformation, induce expression of your recombinant fusion protein from pYES2.1-E or pYC2-E using galactose.

Basic Yeast Molecular Biology

The user should be familiar with basic yeast molecular biology and microbiological techniques. Please refer to *Current Protocols in Molecular Biology*, Unit 13 (Ausubel *et al.*, 1994) and the *Guide to Yeast Genetics and Molecular Biology* (Guthrie and Fink, 1991) for information on preparing yeast media and handling yeast.

Genotype/ Phenotype of INVSc1

The genotype and phenotype of the INVSc1 host strain are provided below.

Genotype: MATα/MATa *his3Δ1*/*his3Δ1 leu2*/*leu2 trp1-289*/*trp1-289 ura3-52*/*ura3-52*

Phenotype: His^-, Leu^-, Trp^-, Ura^-

Note that INVSc1 is a **diploid** strain that is auxotrophic for histidine, leucine, tryptophan, and uracil. The strain will not grow in SC minimal medium that is deficient in histidine, leucine, tryptophan, or uracil. A recipe for preparation of SC minimal medium is provided in the **Appendix**, page 18.

The INVSc1 strain is a suitable strain to use for expression purposes, but should not be used for genetic analyses because it does not sporulate well.

Initiating INVSc1 Culture

To initiate a culture of INVSc1 from the stab provided with the kit, streak a small amount from the stab on a YPD plate (see **Appendix** for recipe, page 19) and incubate at 30°C. Once growth is established, you may check the phenotype of the strain by streaking a single colony on an SC minimal plate supplemented with the appropriate amino acids. INVSc1 will not grow in SC minimal medium that is deficient in histidine, leucine, tryptophan, or uracil.

Be sure to make glycerol stocks of the strain. Store glycerol stocks at -80°C. If you plan to use the strain directly from plates, be sure that the plates are less than 4 days old.

Plasmid Isolation

Isolate plasmid DNA from *E. coli* for yeast transformation using your method of choice. We recommend the S.N.A.P.™ MiniPrep Kit (Catalog no. K1900-01) or the S.N.A.P.™ MidiPrep Kit (Catalog no. K1910-01). Other resin-based methods are suitable.

Positive Control

The pYES2.1-E and pYC2-E are supplied with a corresponding positive control vector (pYES2.1-E/Uni-*lacZ* and pYC2-E/Uni-*lacZ*, respectively) to help you optimize expression conditions for your protein. The gene encoding β-galactosidase is expressed in yeast cells under the control of the *GAL1* promoter. Successful transformation and galactose induction will result in β-galactosidase expression that can be easily assayed (see next page).

Continued on next page

Yeast Transformation, continued

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972). Invitrogen offers the β-Gal Assay Kit (Catalog no. K1455-01) for fast and easy detection of β-galactosidase expression.

Reagents for Yeast Transformation

Many protocols are suitable for the preparation of competent INVSc1 yeast cells. The *S.c.* EasyComp™ Kit (Catalog no. K5050-01) provides a quick and easy method for the preparation of competent yeast cells that can be used immediately or stored frozen for future use. Transformation efficiency is guaranteed at $>10^3$ transformants per µg DNA.

A small-scale yeast transformation protocol is included in the **Appendix** (see page 16) for your convenience. Alternatively, there are published references for other small-scale transformation methods (Gietz *et al.*, 1992; Gietz *et al.*, 1995; Hill *et al.*, 1991; Schiestl and Gietz, 1989).

Transforming Yeast

Use one of the methods described above (or one of your own choosing) to transform your pYES2.1-E or pYC2-E fusion vector into competent INVSc1. We recommend that you include the appropriate control vector (see the previous page) as a positive control for expression and a sample with no DNA as a negative control for transformation.

Select for transformants on SC minimal media lacking uracil (SC-U). Transformants should exhibit the uracil prototrophy. See the **Appendix**, page 18 for a recipe to prepare SC minimal media.

Once you have identified a transformant, be sure to purify the colony and make a glycerol stock for long-term storage.

Maintaining Transformants

Maintain yeast cells containing your pYES2.1-E or pYC2-E fusion vector in SC-U medium containing 2% glucose or 2% raffinose (see the next page).

Note: The growth rate of yeast strains varies with the carbon source. Yeast strains typically exhibit the fastest growth in medium containing glucose.

Expression of Your Recombinant Protein

Introduction

Once you have obtained a transformant containing your pYES2.1-E or pYC2-E fusion vector, you are ready to induce expression of your recombinant fusion protein of interest. This section provides information on how to induce and assay for expression of your protein of interest.

GAL1 Promoter

In INVSc1, transcription from the *GAL1* promoter is repressed in the presence of glucose (West *et al.*, 1984). Removing glucose and adding galactose as a carbon source induces transcription (Giniger *et al.*, 1985). Maintaining cells in glucose gives the most complete repression and the lowest basal transcription of the *GAL1* promoter. Transferring cells from glucose- to galactose-containing medium causes the *GAL1* promoter to become de-repressed and allows transcription to be induced.

Alternatively, cells may be maintained in medium containing raffinose as a carbon source. The presence of raffinose does not repress or induce transcription from the *GAL1* promoter. Addition of galactose to the medium induces transcription from the *GAL1* promoter even in the presence of raffinose. Induction of the *GAL1* promoter by galactose is more rapid in cells maintained in raffinose when compared to those maintained in glucose.

You may choose to grow cells containing your pYES2.1-E or pYC2-E fusion vector in glucose or raffinose depending on how quickly you want to obtain your expressed protein after induction with galactose and on the toxicity of the expressed protein. For more information about expression in yeast, please refer to the *Guide to Yeast Genetics and Molecular Biology* (Guthrie and Fink, 1991).

For a protocol to induce expression of your fusion protein with galactose, proceed to **Time Course of Protein Induction by Galactose** on the next page.

Continued on next page

Expression of Recombinant Protein, continued

Time Course of Protein Induction by Galactose

To induce expression of your protein of interest from the *GAL1* promoter, galactose is added to the medium. For cells that have been maintained in glucose, recombinant fusion protein can be detected in as little as 4 hours after galactose induction. Recombinant fusion protein can be detected in cells that have been cultured in raffinose by 2 hours after galactose induction.

If you are assaying for expression of your recombinant fusion protein for the first time, we recommend that you perform a time course to optimize expression of your recombinant protein (e.g. 0, 4, 8, 12, 16, 24 hours after galactose induction). A standard protocol is provided below to perform a time course experiment. Other protocols are suitable.

1. Inoculate a single colony of INVSc1 containing your pYES2.1-E or pYC2-E fusion vector into 15 ml of SC-U selective medium containing 2% glucose or 2% raffinose. Grow overnight at 30°C with shaking.
2. Determine the OD_{600} of your overnight culture. Calculate the amount of overnight culture necessary to obtain an OD_{600} of 0.4 in 50 ml of *induction medium* (SC-U selective medium containing 2% galactose).

   Example: Assume that the OD_{600} of an overnight culture is 3 OD_{600} per ml. Then, the amount of overnight culture needed to inoculate a 50 ml culture to OD_{600} = 0.4 is

   $$\frac{(0.4\ \text{OD/ml})\ (50\ \text{ml})}{3\ \text{OD/ml}} = 6.67\ \text{ml}$$
3. Remove the amount of overnight culture as determined in Step 2 and pellet the cells at 1500 x g for 5 minutes at room temperature. Discard the supernatant.
4. Resuspend the cells in 50 ml of *induction medium*. Grow at 30°C with shaking.
5. Harvest an aliquot of cells at 0, 4, 8, 12, 16, and 24 hours after addition of cells to the *induction medium*. For each time point, remove 5 ml of culture from the flask and determine the OD_{600} of each sample. You will use this information when assaying for your recombinant fusion protein (see Step 3 on the next page).
6. Centrifuge the cells at 1500 x g for 5 minutes at +4°C.
7. Decant the supernatant. Resuspend cells in 500 µl of sterile water.
8. Transfer cells to a sterile microcentrifuge tube. Centrifuge samples for 30 seconds at top speed in the microcentrifuge.
9. Remove the supernatant.
10. Store the cell pellets at -80°C until ready to use. Proceed to the next section to prepare cell lysates to detect your recombinant protein (see the next page).

Continued on next page

Expression of Recombinant Protein, continued

Detection of Recombinant Fusion Protein

To detect expression of your recombinant fusion protein by western blot (see below), you may use the Anti-V5 or the Anti-His(C-term) antibodies available from Invitrogen (see page viii for ordering information) or an antibody to your protein of interest.

You will also need to prepare a cell lysate from your yeast transformant. A general protocol for small-scale preparation of cell lysates using acid-washed glass beads is provided below for your convenience. Other protocols are suitable. Please refer to *Current Protocols in Molecular Biology*, Unit 13.13 (Ausubel *et al.*, 1994) for more information. For large-scale preparations (culture volumes over 1 liter), see **Scale-up** on the next page.

Materials Needed:

- Breaking buffer (50 mM sodium phosphate, pH 7.4, 1 mM EDTA, 5% glycerol, 1 mM PMSF) (please refer to **Appendix**, page 19 for instructions to prepare the sodium phosphate stock buffer).
- Acid-washed glass beads (0.4-0.6 mm size; Sigma-Aldrich, Catalog no. G8772).

Protocol:

1. You may prepare cell lysates from either frozen cells or fresh cells.

   Reminder: You will need to know the OD_{600} of your cell sample(s) before beginning (see Step 5, previous page).
2. Resuspend fresh or frozen cell pellets in 500 µl of breaking buffer. Centrifuge at 1500 x g for 5 minutes at +4°C to pellet cells.
3. Remove supernatant and resuspend the cells in a volume of breaking buffer to obtain an OD_{600} of 50-100. Use the OD_{600} determined in Step 5, previous page, to calculate the appropriate volume of breaking buffer to use.
4. Add an equal volume of acid-washed glass beads.
5. Vortex mixture for 30 seconds, followed by 30 seconds on ice. Repeat four times for a total of four minutes to lyse the cells. Cells will be lysed by shear force. You can check for the extent of lysis by checking a small aliquot under the microscope.
6. Centrifuge in a microcentrifuge for 10 minutes at maximum speed.
7. Remove supernatant and transfer to a fresh microcentrifuge tube. Assay the lysate for protein concentration using BSA as a standard.
8. Add SDS-PAGE sample buffer to a final concentration of 1X and heat the sample for 5 minutes at 70°C.
9. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your recombinant protein.

Note

If you cloned your PCR product in frame with the C-terminal peptide, this will increase the size of your protein by ~3 kDa.

Continued on next page

Expression of Recombinant Protein, continued

Scale-up of Expression for Purification

Once you have determined the optimal induction time necessary to obtain maximal protein expression, you may increase the protein yield by scaling up the procedure described on page 13. If you plan to use ProBond™ resin to purify your recombinant fusion protein, please see the **Note** below. To prepare cell lysates from culture volumes over 1 liter, we recommend that you use a bead beater (Biospec Products, Bartlesville, OK) to lyse the cells. Please refer to *Current Protocols in Molecular Biology*, Unit 13.13 (Ausubel *et al.*, 1994) for a suitable protocol to lyse cells with a bead beater.

Purification

For help with purification of your recombinant fusion protein, please refer to the ProBond™ Purification System manual. You can download the manual from our Web site (www.invitrogen.com) or request a copy from Technical Services (see page 26).

If you are using another type of resin, please refer to the manufacturer's recommendations.

Appendix

Small-Scale Yeast Transformation

Introduction

A small-scale yeast transformation protocol for routine transformations is provided below. Other protocols are suitable. The *S.c.* EasyComp™ Transformation Kit (Catalog no. K5050-01) is available from Invitrogen for rapid preparation of transformation-competent yeast cells. Please visit our Web site (www.invitrogen.com) or call Technical Service for more information (see page 26).

Materials Needed

Be sure to have the following reagents on hand before starting.

- YPD liquid medium (see **Recipe**, page 19)
- 1X TE (see **Recipe**, page 19)
- 1X LiAc/0.5X TE (see **Recipe**, page 20)
- Denatured salmon sperm DNA (Sigma-Aldrich, Catalog no. D9156)
- Fusion vector construct (or other plasmid DNA to be transformed)
- 1X LiAc/40% PEG-3350/1X TE (See **Recipe**, page 20)
- DMSO
- Selective plates

Protocol

1. Inoculate 10 ml of YPD medium with a colony of INVSc1 and shake overnight at 30°C.
2. Determine the OD_{600} of your overnight culture. Dilute culture to an OD_{600} of 0.4 in 50 ml of YPD medium and grow an additional 2-4 hours.
3. Pellet the cells at 1500 x g and resuspend the pellet in 40 ml 1X TE.
4. Pellet the cells at 1500 x g and resuspend the pellet in 2 ml of 1X LiAc/0.5X TE.
5. Incubate the cells at room temperature for 10 minutes.
6. For each transformation, mix together 1 µg plasmid DNA and 100 µg denatured sheared salmon sperm DNA with 100 µl of the yeast suspension from Step 5.
7. Add 700 µl of 1X LiAc/40% PEG-3350/1X TE and mix well.
8. Incubate solution at 30°C for 30 minutes.
9. Add 88 µl DMSO, mix well, and heat shock at 42°C for 7 minutes.
10. Centrifuge in a microcentrifuge for 10 seconds and remove supernatant.
11. Resuspend the cell pellet in 1 ml 1X TE and re-pellet.
12. Resuspend the cell pellet in 50-100 µl 1X TE and plate on a selective plate.

Note

To calculate the number of yeast cells, assume that 1 OD_{600} unit = ~2.0 x 10^7 yeast cells/ml.

Recipes

LB (Luria-Bertani) Medium and Plates

Composition:

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic if needed.
4. Store at room temperature or at +4°C.

LB agar plates

1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic (50 µg/ml of kanamycin), and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C, in the dark.

SOB Medium (with Kanamycin)

SOB (per liter)

2% Tryptone
0.5% Yeast Extract
0.05% NaCl
2.5 mM KCl
10 mM $MgCl_2$

1. Dissolve 20 g tryptone, 5 g yeast extract, and 0.5 g NaCl in 950 ml deionized water.
2. Make a 250 mM KCl solution by dissolving 1.86 g of KCl in 100 ml of deionized water. Add 10 ml of this stock KCl solution to the solution in Step 1.
3. Adjust pH to 7.5 with 5 M NaOH and add deionized water to 1 liter.
4. Autoclave this solution, cool to ~55°C, and add 10 ml of sterile 1 M $MgCl_2$. You may also add kanamycin to 50 µg/ml.
5. Store at +4°C. Medium is stable for only ~1 month.

Continued on next page

Recipes, continued

SC Minimal Medium and Plates

SC is synthetic minimal defined medium for yeast.

0.67% yeast nitrogen base (**without** amino acids but **with** ammonium sulfate)
2% carbon source (i.e. glucose or raffinose)
0.01% (adenine, arginine, cysteine, leucine, lysine, threonine, tryptophan, uracil)
0.005% (aspartic acid, histidine, isoleucine, methionine, phenylalanine, proline, serine, tyrosine, valine)
2% agar (for plates)

1. Dissolve the following reagents in 900 ml deionized water (800 ml if preparing medium containing raffinose). **Note:** We make medium and plates as we need them and weigh out each amino acid. Many researchers prepare 100X solutions of each amino acid that they need.

Reminder: Omit uracil to make selective plates for growing pYES2.1-E or pYC2-E fusion vector transformants.

6.7 g Yeast Nitrogen Base	**0.1 g each**	**0.05 g each**
	adenine	aspartic acid
	arginine	histidine
	cysteine	isoleucine
	leucine	methionine
	lysine	phenylalanine
	threonine	proline
	tryptophan (W)	serine
	uracil (U)	tyrosine
		valine

2. If you are making plates, add the agar after dissolving the reagents above.
3. Autoclave at 15 psi, 121°C for 20 minutes.
4. Cool to 50°C and add 100 ml of filter-sterilized 20% glucose or 200 ml of filter-sterilized 10% raffinose.
5. Pour plates and allow to harden. Invert the plates and store at +4°C. Plates are stable for 6 months.

Induction Medium

If you are making induction medium, follow Steps 1-3 above except dissolve the reagents in 800 ml of deionized water. Cool the medium to 50°C and add 100 ml of filter-sterilized 20% galactose and 100 ml of filter-sterilized 10% raffinose to the medium. Note: Raffinose is included to increase growth rate.

When making stock solutions of raffinose, do not autoclave the stock solution. Autoclaving the solution will convert the raffinose to glucose. Filter-sterilize the stock solution.

Continued on next page

Recipes, continued

YPD

Yeast Extract Peptone Dextrose Medium (1 liter)

1% yeast extract
2% peptone
2% dextrose (D-glucose)

1. Dissolve the following in 1000 ml of water:

   10 g yeast extract
   20 g peptone
   20 g dextrose (see note below if making plates)

2. Optional: Add 20 g agar, if making plates.
3. Autoclave for 20 minutes on liquid cycle.
4. Store medium at room temperature or cool the medium and pour plates. The shelf life is approximately one to two months.

Note: If making plates, omit dextrose from Step 1. Autoclaving agar and dextrose together will cause the dextrose to caramelize. Prepare a separate stock solution of 20% dextrose and autoclave or filter-sterilize. After the YPD broth (900 ml volume) has been autoclaved, add 100 ml of 20% dextrose to the medium.

0.1 M Sodium Phosphate, pH 7.4

Before beginning, have the following reagents on hand.

- Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_2O$; Sigma-Aldrich S9638)
- Sodium phosphate, dibasic ($Na_2HPO_4 \cdot 7H_2O$; Sigma-Aldrich S9390)

1. Prepare 100 ml of 1 M $NaH_2PO_4 \cdot H_2O$ by dissolving 13.8 g in 90 ml of deionized water. Bring volume up to 100 ml. Filter-sterilize.
2. Prepare 100 ml of 1 M $Na_2HPO_4 \cdot 7H_2O$ by dissolving 26.81 g in 90 ml of deionized water. Bring volume up to 100 ml. Filter-sterilize.
3. For 1 liter of 0.1 M sodium phosphate, pH 7.4, mix together 22.6 ml of 1 M NaH_2PO_4 and 77.4 ml of 1 M Na_2HPO_4. Bring up the volume to 1 liter with sterile water.
4. Filter-sterilize and store at room temperature.

10X TE

100 mM Tris, pH 7.5
10 mM EDTA

1. For 100 ml, dissolve 1.21 g of Tris base and 0.37 g of EDTA in 90 ml of deionized water.
2. Adjust the pH to 7.5 with concentrated HCl and bring the volume up to 100 ml.
3. Filter-sterilize and store at room temperature.

Alternatively, you can make the solution using 1 M Tris-HCl, pH 7.5 and 0.5 M EDTA, pH 8.0.

1X TE

10 mM Tris, pH 7.5
1 mM EDTA

Dilute 10X TE 10-fold with sterile water.

Continued on next page

Recipes, continued

10X LiAc

1 M Lithium Acetate, pH 7.5

1. For 100 ml, dissolve 10.2 g of lithium acetate in 90 ml of deionized water.
2. Adjust pH to 7.5 with dilute glacial acetic acid and bring up the volume to 100 ml.
3. Filter-sterilize and store at room temperature.

1X LiAc

100 mM Lithium Acetate, pH 7.5

Dilute 10X LiAc solution 10-fold with sterile, deionized water.

1X LiAc/0.5X TE

100 mM Lithium Acetate, pH 7.5
5 mM Tris-HCl, pH 7.5
0.5 mM EDTA

1. For 100 ml, mix together 10 ml of 10X LiAc and 5 ml of 10X TE.
2. Add deionized water to 100 ml.
3. Filter-sterilize and store at room temperature.

1X LiAc/40% PEG-3350/1X TE

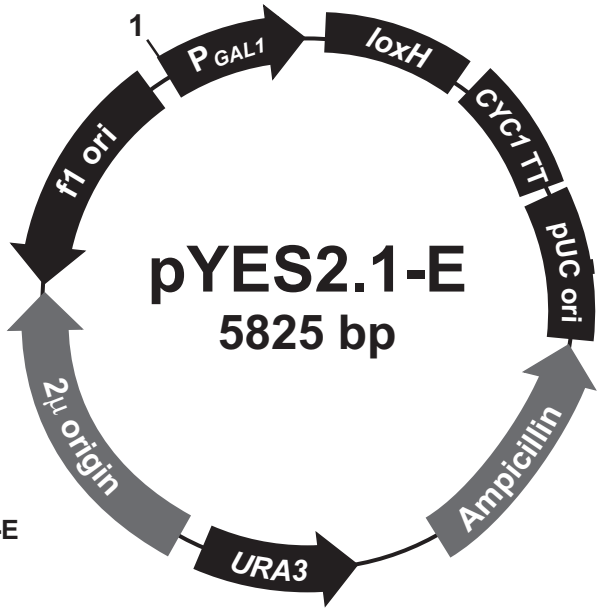
100 mM Lithium Acetate, pH 7.5
40% PEG-3350
10 mM Tris-HCl, pH 7.5
1 mM EDTA

1. Prepare solution immediately prior to use. For 100 ml, mix together 10 ml of 10X LiAc, 10 ml of 10X TE, and 40 g of PEG-3350.
2. Add deionized water to 100 ml and dissolve the PEG. You may have to heat the solution to fully dissolve the PEG.
3. Autoclave at 121°C, 15 psi for 20 minutes. Store at room temperature.

Maps of pYES2.1-E and pYC2-E

pYES2.1-E Map

The map below shows the features of pYES2.1-E (5825 bp). **The complete sequence of the vector is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (page 26).**



Features of pYES2.1-E
5825 nucleotides

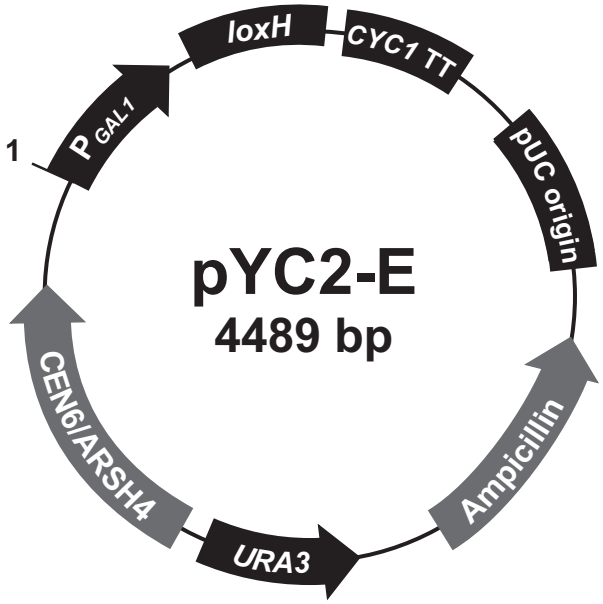
GAL1 promoter: bases 1-451
GAL1 promoter priming site: bases 414-437
T7 promoter priming site: bases 475-494
loxH site: bases 507-540
CYC1 transcriptional termination region: bases 570-823
CYC1 reverse priming site: bases 587-605
pUC origin: bases 1007-1680
Ampicillin resistance gene (bla): base 1825-2685 (C)
URA3 gene: 2703-3810 (C)
2 micron origin: bases 3814-5285
f1 origin: bases: 5353-5808
(C) = complementary

Continued on next page

Maps of pYES2.1-E and pYC2-E, continued

pYC2-E Map

The map below shows the features of pYC2-E (4489 bp). **The complete sequence of the vector is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (page 26).**



Features of pYC2-E
4489 nucleotides

GAL1 promoter: bases 1-451
GAL1 Forward priming site: bases 414-437
T7 promoter priming site: bases 475-494
loxH site: bases 507-540
CYC1 transcriptional termination region: bases 570-823
CYC1 Reverse priming site: bases 587-605
pUC origin: bases 1007-1680
Ampicillin resistance gene: bases 1825-2685 (C)
URA3 gene: bases 2703-3810 (C)
CEN6/*ARSH4*: bases 3823-4341
f1 origin:bases 4342-4447
(C) = complementary strand

Features of pYES2.1-E and pYC2-E

Features

The important elements of pYES2.1-E and pYC2-E are described in the following table. All features have been functionally tested.

Feature	Benefit
GAL1 promoter	Permits galactose-inducible expression of genes cloned into pYES2/CT (West *et al.*, 1984)
GAL1 forward priming site	Allows sequencing through the insert
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
loxH site	Allows recombination between the donor vector and pYES2.1-E and pYC2-E (Hoess *et al.*, 1982)
CYC1 transcription termination signal	Permits efficient termination and stabilization of mRNA
CYC1 reverse priming site	Allows sequencing through the insert
pUC origin	Allows maintenance and high copy replication in *E. coli*
Ampicillin resistance gene	Allows selection of transformants in *E. coli*
URA3 gene	Permits selection of yeast transformants in uracil-deficient medium
2µ origin (pYES2.1-E)	Permits episomal maintenance and high copy replication in yeast
CEN6/*ARSH4* sequence (pYC2-E)	Permits non-integrative centromeric maintenance and low copy replication in yeast (Sikorski and Hieter, 1989)
f1 origin	Allows rescue of single-stranded DNA

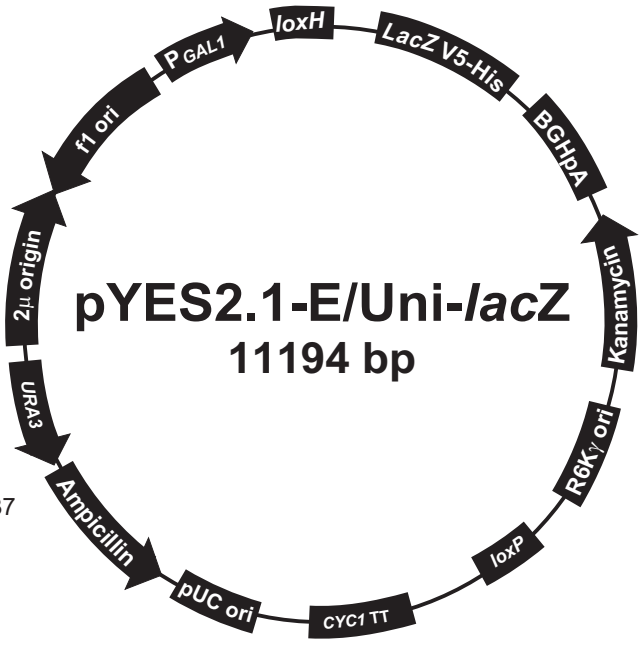
Map of pYES2.1-E/Uni-*lacZ*

Description

pYES2.1-E/Uni-*lacZ* is a 11194 bp control vector expressing β-galactosidase. The *lacZ* gene was amplified and TOPO® Cloned into pUni/V5-His/Gene-TOPO®. The resulting vector was recombined with pYES2.1-E using Cre recombinase to create pYES2.1-E/Uni-*lacZ*. **Note**: pUni/V5-His/Gene-TOPO® is similar to pUni/V5-His-TOPO® TA except that it contains additional DNA between the TOPO® Cloning site and the V5 epitope.

Map of Control Vector

The figure below summarizes the features of the pYES2.1-E/Uni-*lacZ* vector. **The complete nucleotide sequence for pYES2.1-E/Uni-*lacZ* is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 26).**



Features of pYES2.1-E/Uni-*lacZ*
11194 nucleotides

GAL1 promoter: bases 1-451
GAL1 forward priming site: bases 414-437
T7 promoter priming site: bases 475-494
loxH site: bases 507-540
lacZ ORF: bases 561-3617
V5 epitope: bases 3630-3671
6xHis tag: bases 3681-3698
pUni Reverse priming site: bases 3760-3781
BGH polyadenylation region: bases 3779-3987
T7 transcriptional termination region: bases 4002-4130
Kanamycin resistance gene: bases 4309-5103 (C)
kan promoter region: bases 5104-5241 (C)
R6Kγ origin: bases 5464-5855
pUni Forward priming site: bases 5823-5841
loxP site: bases 5876-5909
CYC1 transcription termination region: bases 5939-6192
CYC1 Reverse priming site: bases 5956-5974
pUC origin: bases 6376-7049
Ampicillin resistance gene (bla): bases 7194-8054 (C)
URA3 gene: bases 8072-9179 (C)
2 micron origin: bases 9183-10654
f1 origin: bases 10722-11177
(C) = complementary strand

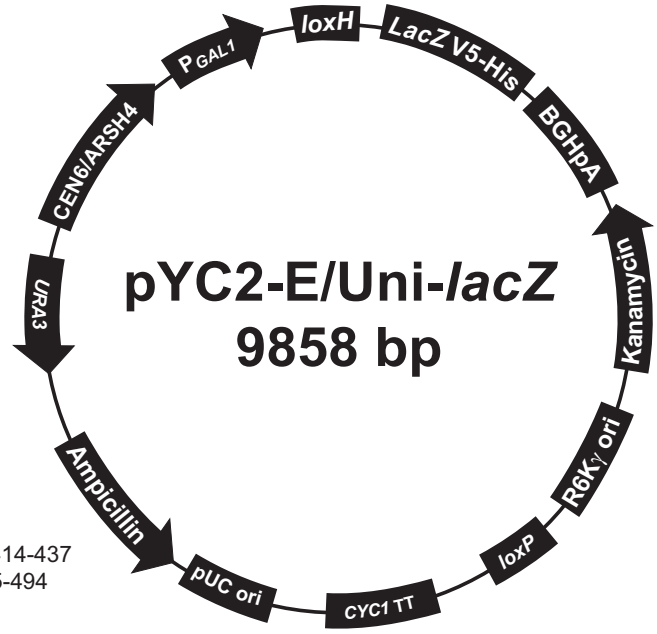
Map of pYC2-E/Uni-*lacZ*

Description

pYC2-E/Uni-*lacZ* is a 9858 bp control vector expressing β-galactosidase. The *lacZ* gene was amplified and TOPO® Cloned into pUni/V5-His/Gene-TOPO®. The resulting vector was recombined with pYC2-E using Cre recombinase to create pYC2-E/Uni-*lacZ*.
Note: pUni/V5-His/Gene-TOPO® is similar to pUni/V5-His-TOPO® TA except that it contains additional DNA between the TOPO® Cloning site and the V5 epitope.

Map of Control Vector

The figure below summarizes the features of the pYC2-E/Uni-*lacZ* vector. **The complete nucleotide sequence for pYC2-E/Uni-*lacZ* is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 26).**



Features of pYC2-E/Uni-*lacZ*
9858 nucleotides

GAL1 promoter: bases 1-451
GAL1 Forward priming site: bases 414-437
T7 promoter priming site: bases 475-494
loxH site: bases 507-540
lacZ ORF: bases 561-3617
V5 epitope: bases 3630-3671
6xHis tag: bases 3681-3698
pUni Reverse priming site: bases 3760-3781
BGH polyadenylation region: bases 3779-3987
T7 transcription termination region: bases 4002-4130
Kanamycin resistance gene (ORF): bases 4309-5103 (C)
kan promoter: bases 5104-5241 (C)
R6Kγ origin: bases 5464-5855
pUni Forward priming site: bases 5823-5841
loxP site: bases 5876-5909
CYC1 transcriptional termination region: bases 5939-6192
CYC1 Reverse priming site: bases 5956-5974
pUC origin: bases 6576-7049
Ampicillin resistance gene (bla) ORF: bases 7181-8041 (C)
URA3 gene: bases 8072-9179 (C)
CEN6/*ARS4*: bases 9192-9710
f1 origin: bases 9711-9816
(C) = complementary strand

Technical Service

Web Resources

Visit the Invitrogen Web site at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical service contact information
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Information for European Customers	The INVSc1 yeast strain is genetically modified and carries the auxotrophic reporter gene *HIS3*. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Product Qualification

Vectors

pYES2.1-E, pYES2.1-E/Uni-*lacZ*, pYC2-E, and pYC2-E/Uni-*lacZ* are qualified by restriction digest. The restriction enzymes and the expected fragments are listed below.

Restriction Enzyme	pYES2.1-E	pYES2.1-E/ Uni-*lacZ*	pYC2-E	pYC2-E/ Uni-*lacZ*
*Apa*L I	4579 bp 1246 bp	Not tested	3243 bp 1246 bp	Not tested
*Eco*R V	Not tested	4634 bp, 4103 bp 2457 bp	Not tested	4634 bp 2767 bp 2457 bp
*Hin*d III	5725 bp	6120 bp 5074 bp	4489 bp	5074 bp 4784 bp
Drd I	Not tested	Not tested	4489 bp	Not tested
*Bam*H I	Not tested	Not tested	Not tested	9858 bp
Pme I	5825 bp	Not tested	4489 bp	Not tested

Primers

The T7 Forward Sequencing primer has been lot-qualified by DNA sequencing experiments using the dideoxy chain termination technique.

INVSc1

The INVSc1 yeast strain is tested for growth on YPD medium.

Cre Recombinase

Purity: >95% homogeneity

Endonuclease activity: Negative

Exonuclease activity: Negative

Functional Assay: Cre recombinase is qualified using the assay on page 5 of this manual. The donor vector is pUni/*lacZ* and the acceptor vector is pcDNA3.1-E. Five microliters of the recombination reaction is transformed into 50 µl One Shot® TOP10 competent *E. coli* using the protocol on page 6. Twenty-five microliters of the transformation reaction is plated on LB plates containing 50 µg/ml kanamycin and X-gal (performed in duplicate). One microliter of Cre recombinase should yield >500 blue, kanamycin-resistant transformants.

One Shot® TOP10 Competent *E. coli*

All competent cells are qualified as follows:

- Cells are tested for transformation efficiency using the control plasmid included in the kit. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be ~1 x 10^9 cfu/µg DNA for chemically competent cells.
- To verify the absence of phage contamination, 0.5-1 ml of competent cells are added to LB top agar and poured onto LB plates. After overnight incubation, no plaques should be detected.
- Untransformed cells are plated on LB plates 100 µg/ml ampicillin, 25 µg/ml streptomycin, 50 µg/ml kanamycin, or 15 µg/ml chloramphenicol to verify the absence of antibiotic-resistant contamination.

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